Vitis 14, 14—19 (1975)

Botany School, University of Melbourne, Parkville, Victoria, Australia

Scanning electron microscopy of the developmental stages of the Sultana inflorescence

by

P. B. Scholefield and R. C. Ward

Rasterelektronenmikroskopische Untersuchungen über die Entwicklungsstadien der Sultana-Infloreszenz

Zusammenfassung. — Die Entwicklung der Infloreszenzprimordien von Sultana wird aufgrund rasterelektronenmikroskopischer Beobachtungen beschrieben. Die angewandte Technik ist einfach und erfordert keine langwierige Vorbehandlung des Untersuchungsmaterials. Das hohe Auflösungsvermögen und die große Tiefenschärfe des Rasterelektronenmikroskopes erleichtern das Verständnis der genauen Infloreszenzentwicklung. Die ersten Anzeichen für die Differenzierung von Blütenteilen wurden bei Sultana unter australischen Bedingungen im Frühjahr festgestellt.

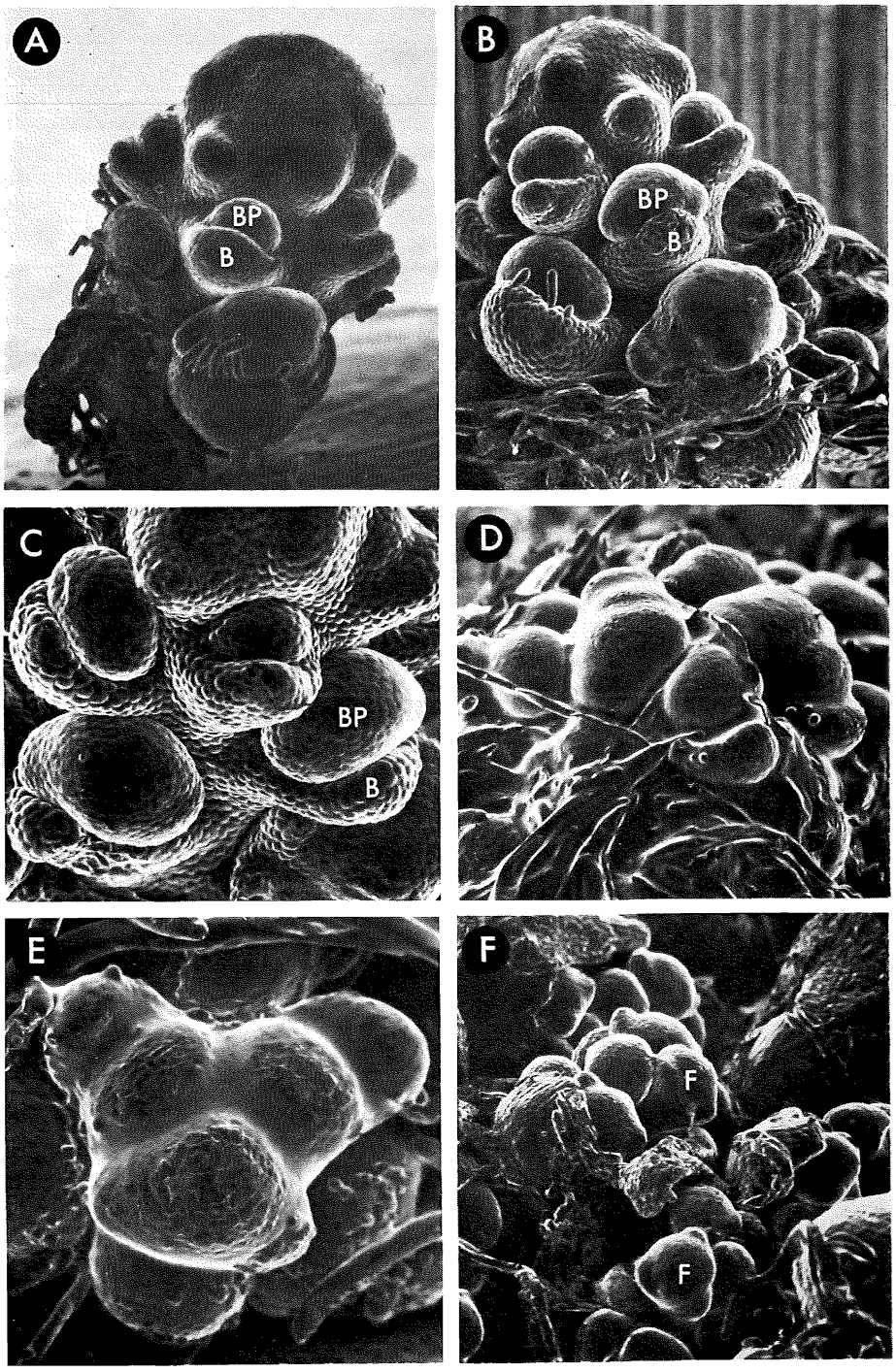
Introduction

The development of the Sultana (*Vitis vinifera* L.) inflorescence from initiation to flowering has been investigated previously by Barnard and Thomas (1933), and Winkler and Shemsettin (1937). In Southern Australia, the inflorescence primordium is initiated in an axillary bud in late November. It then develops rapidly until the onset of organic dormancy in the following January. Little further development occurs in autumn and winter until early-mid August when the inflorescence primordium begins to increase in size and complexity (May 1964). The buds burst in early September and the flowers differentiate shortly after. Floral parts develop until anthesis in November.

Using other cultivars, Alleweldt and Balkema (1965), Alleweldt and Ilter (1969), and Agaoglu (1971), have reported floral parts on the inflorescence primordium in late autumn of the season of initiation.

Fig. 1: (A) May 6th; Inflorescence primordium from a dormant bud. No floral development is evident. × 130. (B) August 2nd; Inflorescence primordium from a dormant bud. Note the comparable size and stage of development with May 6th primordium. × 120. (C) August 2nd; Portion of an inflorescence primordium. × 185. (D) August 28th; Division and expansion of the inflorescence primordium at the time of budburst. × 115. (E) August 28th; Three floral apices with bracts. Calyx not yet differentiated. × 225. (F) September 13th; Group of individual flowers with calyx differentiated. × 95.
BP = branch primordium, B = bract, F = flower.

(A) 6. Mai; Infloreszenz-Primordium einer schlafenden Knospe. Keine Blütenbildung sichtbar. Vergrößerung 200 ×. (B) 2. August; Infloreszenz-Primordium einer schlafenden Knospe. Größe und Entwicklungsstadium sind dem am 6. Mai aufgenommenen Primordium vergleichbar. Vergrößerung 120 ×. (C) 2. August; Teil eines Infloreszenz-Primordiums. Vergrößerung 185 ×. (D) 28. August; Teilung und Vergrößerung des Infloreszenz-Primordiums zur Zeit des Knospenaustriebs. Vergrößerung 115 ×. (E) 28. August; Drei Blütenapices mit Brakteen. Kelch noch nicht differenziert. Vergrößerung 225 ×. (F) 13. September; Gruppe von Einzelblüten mit differenziertem Kelch. Vergrößerung 95 ×.
BP = Primordium einer Verzweigung, B = Braktee, F = Blüte.


A
BP
B
B
BP
B
C
BP
B
D
E
F
F
F

In a recent review, Pratt (1971) commented on the need for clarification of the time of flower initiation.

Previous workers have used transverse and longitudinal sections of inflorescences or flowers and, in some cases, the interpretation of the micrographs is difficult.

Sattler (1968) described a method of staining and examining whole specimens under ethyl alcohol, which allowed a three dimensional analysis.

The Scanning Electron microscope gives similar results but with greater accuracy. Troughton and Donaldson (1972) used it to survey a wide range of plant structures.

Materials and Methods

Sultana vines, forty-five years old, growing in the irrigated vineyard of C.S.I.R.O., Division of Horticultural Research, Merbein, Australia, were used as a source of material. Samples of inflorescence primordia or inflorescences were taken from buds at nodes eight to eleven of mature shoots on May 6th and August 3rd, and from shoots arising from these bud positions after budburst on August 28th and 30th, September 6th, 13th, and 28th; and October 12th, 1973. Budburst commenced on August 28th.

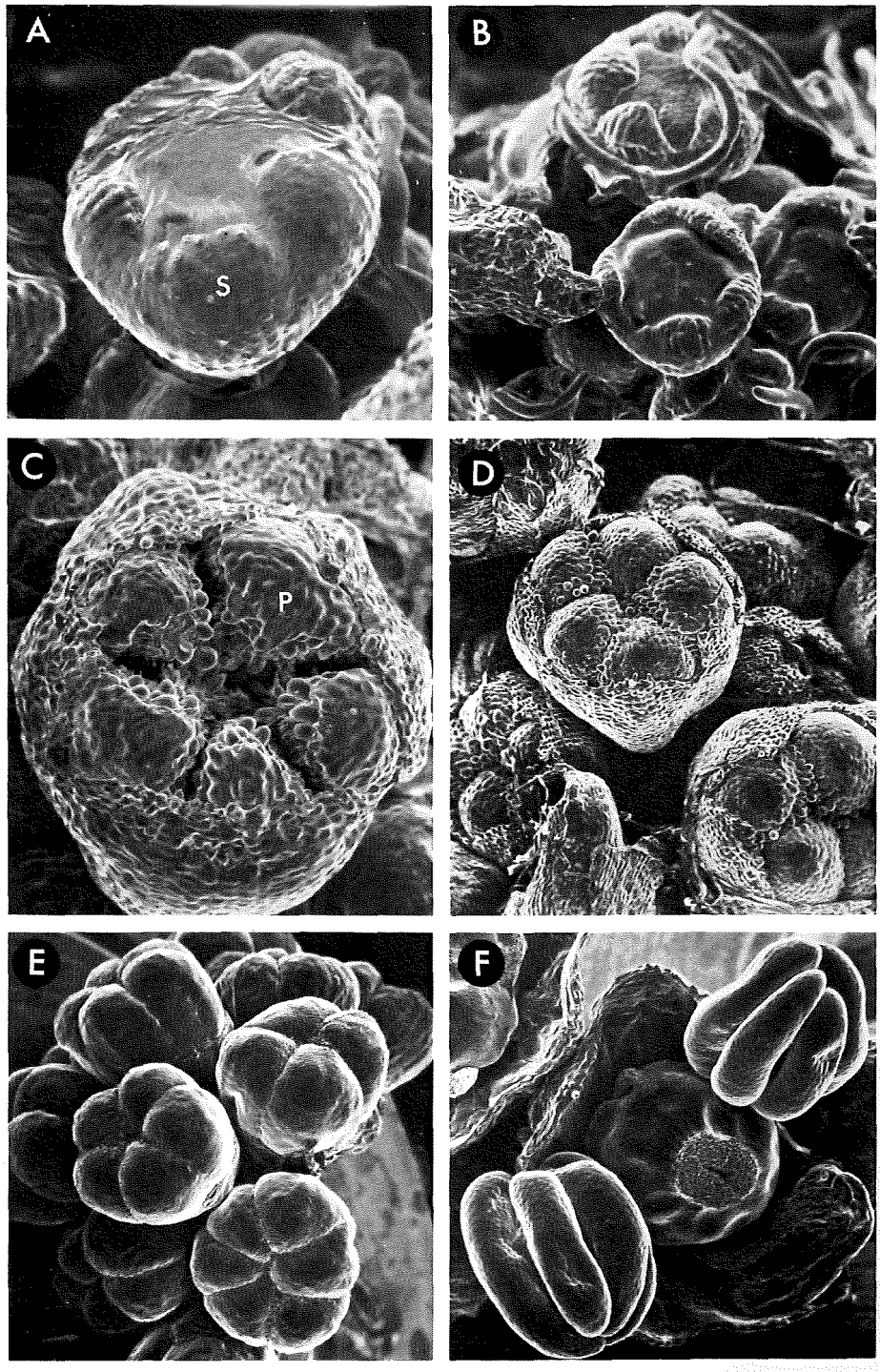
Portions of the dormant shoots from May 6th, and August 3rd were kept in polythene bags in a cool room at 3 °C and the inflorescence primordia dissected under a binocular microscope immediately prior to examination. At all other sampling dates, the inflorescence or inflorescence portion was taken in the field, fixed in a solution of 3 percent (v : v) glutaraldehyde in 0.1M Sodium cacodylate buffer (pH 6.8), and stored in this solution at 3 °C.

The specimens were placed on, or attached with DAG 915[1]) to an aluminium SEM stub. Large specimens were placed on a fine sewing needle mounted, point up, on the stub.

Mounted fresh, specimens were placed directly into the chamber of a Cambridge Stereoscan S4-10, and examined using a very low electron gun accelerating voltage of 3kV (Troughton and Donaldson, pers. comm.). This allowed each specimen to be examined for at least five minutes, reducing damage from the electron beam, and

[1]) DAG 915, silver in m.i.b.k. Acheson Colloids Company, Prince Rock, Plymouth, England.

Fig. 2: (A) September 13th; Single flower with the calyx differentiated. × 250. (B) September 13th; Group of flowers at a more advanced stage than (A). × 125. (C) September 28th; Flower, showing the petals almost closed together. × 100. (D) September 28th; Group of flowers. Note the interlocking cells around the edge of petals. × 45. (E) October 12th; Group of mature flowers. Note the flowers with 4, 5 and 6 petals. × 18. (F) October 12th; Flower with the calyptra and 3 stamens removed. × 52.
S = sepal, P = petal.
(A) 13. September; Einzelblüte mit differenziertem Kelch. Vergrößerung 250 ×. (B) 13. September; Gruppe von Einzelblüten in weiter fortgeschrittenem Entwicklungsstadium als in (A). Vergrößerung 125 ×. (C) 28. September; Blüte, mit fast völlig zusammengeschlossenen Kronblättern. Vergrößerung 100 ×. (D) 28. September; Gruppe von Blüten. Beachte die ineinander verzahnten Zellen am Rande der Blütenblätter. Vergrößerung 45 ×. (E) 12. Oktober; Gruppe von voll entwickelten Blüten. Beachte die Blüten mit 4, 5 und 6 Blütenblättern. Vergrößerung 18 ×. (F) 12. Oktober; Blüte, an der Käppchen und 3 Staubblätter entfernt wurden. Vergrößerung 52 ×.
S = Kelchblatt, P = Kronblatt.


A
S
B
C
P
D
E
F

enabling surface detail to be resolved until gross distortion, due to the vacuum, occurred.

At least ten specimens were examined for each sampling date.

Results and Discussion

Scanning electron micrographs showing inflorescence development from May 6th to October 12 th are presented in Figs. 1 and 2.

The structure of the inflorescence primordium in the dormant buds (Fig. 1 A, B, C) correlates with the longitudinal sections of Barnard and Thomas (1933) and Winkler and Shemsettin (1937), but the method allows greater appreciation of the spatial arrangement of the component parts.

The inflorescence primordia in Fig. 1 A and B are structurally similar, indicating little development between May 6th and August 3rd. They consist of many growing apices, each subtended by a bract. In the development of the inflorescence primordium, these apices divide many times to become a branch of the inflorescence with many flowers. For this reason, the growing apex in the inflorescence primordium will be called a branch primordium (BP).

Differentiation of flowers is not evident on May 6th or August 3rd. This agrees with Barnard and Thomas (1933) and Winkler and Shemsettin (1937) but is contrary to the findings of Alleweldt and Balkema (1965), Alleweldt and Ilter (1969), and Agaoglu (1971). Barnard and Thomas (1933) reported that flower differentiation occurred over the whole inflorescence almost simultaneously. As a Sultana inflorescence often consists of a thousand flowers, it is very difficult to imagine an inflorescence primordium branched to this extent in a dormant bud. This would be the case if flower differentiation began in autumn.

The basal branch primordium of Fig. 1 B has divided, giving several subbranches subtended by two bracts. It is possible that Alleweldt and Balkema (1965), Alleweldt and Ilter (1969), and Agaoglu (1971) may have mistaken a section of this type of structure for a flower. However, more studies are required on a series of cultivars under different climatic conditions to clarify this matter.

At budburst, rapid growth and division of the inflorescence primordium occurs (Fig. 1 D). Barnard and Thomas (1933) and May and Antcliff (1973) show sections of floral apices divided into three. A similar structure is shown in Fig. 1 E. Further development of these flower primordia give the flattened structures shown in Fig. 1 F on September 13th, with sepals beginning to form. Thus, individual flowers with floral parts are first observed on September 13th.

The development to complete flowers is shown in Fig. 2 A—F.

The new method, described here, is simple and provides a means for answering the many problems still unsolved in the ontogeny and development of the grape inflorescence.

Summary

Development of the inflorescence primordium of Sultana, as observed in the Scanning Electron Microscope (SEM), is described. The technique is simple and requires no elaborate tissue preparation. Interpretation of inflorescence development is easy and precise because of the resolution and depth of field of the SEM. The first evidence of differentiation of floral parts was observed in spring for Sultana under Australian conditions.

Acknowledgements

The authors gratefully acknowledge the following support: Professor T. C. CHAMBERS for SEM facilities; Dr. J. TROUGHTON and Ms. L. A. DONALDSON for advice on SEM techniques; Drs. P. MAY and T. F. NEALES for invaluable discussions and Mr. E. A. LAWTON and Ms. B. WRIGHT for photographic assistance. P. B. SCHOLEFIELD was in receipt of a studentship from the Dried Fruits Research Committee.

Literature Cited

AGAOGLU, Y. S., 1971: A study on the differentiation and the development of floral parts in grapes (*Vitis vinifera* L. var.). Vitis **10**, 20—26.

ALLEWELDT, G. and BALKEMA, G. H., 1965: Über die Anlage von Infloreszenz- und Blütenprimordien in den Winterknospen der Rebe. Z. Acker- und Pflanzenbau **123**, 59—74.

ALLEWELDT, G. and ILTER, E., 1969: Untersuchungen über die Beziehungen zwischen Blütenbildung und Triebwachstum bei Reben. Vitis **8**, 286—313.

BARNARD, C. and THOMAS, J. E., 1933: Fruit bud studies. — II The Sultana: Differentiation and development of the fruit buds. J. Counc. Sci. Ind. Res. Austral. **6**, 285—294.

MAY, P., 1964: Über die Knospen- und Infloreszenzentwicklung der Rebe. Wein-Wiss. **19**, 457—485.

— — and ANTCLIFF, A. J., 1973: The fruitfulness of grape buds I. Measuring bud fruitfulness on forced single-node cuttings. Ann. Amélior. Plantes **23**, 1—12.

PRATT, C., 1971: Reproductive anatomy in cultivated grapes — a review. Amer. J. Enol. Vitic. **22**, 92—109.

SATTLER, R., 1968: A technique for the study of floral development. Can. J. Bot. **46**, 720—722.

TROUGHTON, J. and DONALDSON, L. A., 1972: Probing Plant Structure. A. H. and A. W. Reed Ltd., Wellington N. Z., 116 p.

WINKLER, A. J. and SHEMSETTIN, E. M., 1937: Fruit-bud and flower formation in the Sultanina grape. Hilgardia **10**, 589—611.

Eingegangen am 7. 10. 1974

P. B. SCHOLEFIELD
CSIRO
Division of Hort. Res.
Merbein, Victoria 3505
Australia